Cosmetics Europe
the personal care association

COSMETICS EUROPE:

GUIDELINES ON MICROBIAL QUALITY MANAGEMENT (MQM)

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1. INTRODUCTION

The purpose of these guidelines is to help those concerned with the production, development and control of cosmetics and toiletries in the maintenance of product quality. These guidelines should not, however, be regarded as definitive. Many manufacturers may well have developed other equally effective techniques which achieve results comparable with those obtained by the procedures suggested in these guidelines.

Microbial Quality Management (MQM) is a process designed to ensure that cosmetic and toiletry companies only manufacture products which conform to specifications and are, therefore, microbiologically safe for customers to use.

An MQM strategy encompasses correct product development to ensure customer safety, supplier quality management and operation of Good Manufacturing Practices in production to prevent microbiological problems occurring. Essential to the implementation of effective MQM is the requirement for a microbial awareness education and training programme for all levels of employees.

2. PRODUCT DEVELOPMENT

2.1 PRODUCT PRESERVATION

The function of preservatives is for consumer protection and prevention of spoilage during normal and reasonably foreseeable product use; preservatives should not be used in lieu of good production hygiene. Control of microbial contamination during production is attained by understanding the causes and eliminating them.

It must be emphasised that preservatives cannot be chosen satisfactorily on theoretical grounds and require *in situ* determination of their efficacy by microbiological challenge tests during product development.

2.2 DEVELOPMENT OF SUITABLE FORMULATIONS

Whenever possible, the formulator should be encouraged to develop formulations which are incapable of supporting microbial growth, hence reducing the need for the addition of a preservative. However, if a preservative is shown to be necessary, it should be selected at an early stage in the development of a product and be considered an integral part of the formulation.

Water is essential for microbial growth and a preservative system should have solubility and partition characteristics so that it is available at an effective concentration in the aqueous phase of a multi-phase system.

A preservative system should be effective against a broad spectrum of micro-organisms and safe at the concentration used. Combinations of preservatives can sometimes be more effective than individual compounds. Temperature, light and prolonged storage stability are important. The preservative system should be effective in low concentration and at the pH of the formulation, and should be compatible with other product constituents.

The packaging should be designed to restrict the ingress of contaminants and also to avoid the formation of condensation water from the product on inner surfaces as this can encourage microbial growth. Inactivation of preservative systems by the container and diffusion through it should be considered.

The inclusion of easily biodegradable raw materials in a formulation makes preservation more important since these raw materials will be more readily utilised as nutrients by contaminating micro-organisms.

Any changes in the raw material specifications of a product formulation may influence the preservative capacity of the product. In such cases, further challenge testing should be considered.

During the manufacture of some products, the preparation of pre-mixes may be required. Aqueous pre-mixes should contain preservatives and should be subject to storage limitations and regular monitoring. To determine their susceptibility to microbiological contamination, they should be subjected to a challenge test.

2.3 CHALLENGE TESTING

If the challenge test is considered to be necessary according to section 2.2 (see above), it should be carried out by a suitably qualified person on the complete formulation in its pack, even though preliminary tests have been performed on this formulation during its development.

In principle, the sample is inoculated with a variety of relevant micro-organisms, bacteria, yeast and moulds, some of which may be pathogenic or cause spoilage. The inoculated product is then tested for viability of the micro-organisms at various periods as long as considered necessary.

3. RAW MATERIALS

Raw materials can contribute a significant level of microbial contamination to the finished product. It should be the aim of both the product and raw materials manufacturer to provide materials low in microbial contamination and free from harmful micro-organisms.

Water is one of the major raw materials used in the formulation of cosmetic and toiletry products and one which, in certain circumstances, may be populated by large numbers of micro-organisms. This may present a distinct hazard to the microbiological stability of finished products. Therefore, steps must be taken to ensure that water, used as an ingredient or for processing, is regularly monitored and, where necessary, appropriate treatment given.

4. CONTROL AND ASSESSMENT OF BULK AND FINISHED PRODUCT

4.1 BULK STORAGE OF PRODUCT

This should be limited by the susceptibility of the product to contamination. Bulk product may be more susceptible than in pack form.

4.2 SAMPLING LEVEL

All products should be sampled at a level appropriate to the anti-microbial activity of the formulation as determined by challenge test and production experience. Any new product should have this level of testing raised for an initial period of at least three months to determine its microbiological profile under normal production conditions.

5. GOOD MANUFACTURING PRACTICE

In order to conform to Good Manufacturing Practice conditions, reference should be made to the Cosmetic Good Manufacturing Guidelines developed by Colipa.

6. DOCUMENTATION

Adequate records should be maintained for all aspects of microbiological testing during the development and manufacture of each product and for all control procedures used at the manufacturing plant.

7. RECOMMENDED MICROBIAL LIMITS AND METHODS FOR FINISHED PRODUCTS

7.1 GENERAL OVERVIEW

It must be the aim of all manufacturers to ensure that their products are safe in all respects under normal and reasonably foreseeable conditions of use.

The planning and execution of MQM testing procedures and the interpretation of results call for special skills. If these are not available within the company, experts should be consulted.

Contaminating micro-organisms may be harmful to the consumer and may cause spoilage of the product. It is, therefore, necessary to limit them. This can be achieved by:

- the use of good plant hygiene and manufacturing practices;
- ensuring that the products are adequately preserved against microbial growth;
- applying microbial limits designed to ensure safe products.

The manufacturer must bear in mind that, when an individual test is made, contaminating micro-organisms may be multiplying, static or decreasing in number. The use of preservatives will help to ensure that products do not allow the growth of micro-organisms. Preservatives are no substitute for good plant hygiene and it must be remembered that their use involves balancing levels which are effective against those which might cause problems to the user. Preservatives should be chosen during development of a product using challenge test procedures.

The methods and specifications detailed below are intended to be used as reference tests and are not intended to be normal quality control tests or specifications. Companies may continue to apply their own internal control specifications and validated test methods, together with an appropriate level of MQM, to satisfy themselves that they produce products which comply with the criteria specified in this document when tested by the methods detailed.

The specification is intended to apply to the product in an unopened original package recognised as intact.

The results of a test should be recorded in a report. This report should cover:

- the identification of the samples
    - product type
    - brand name
    - manufacturer
    - batch number
    - date and place of sampling
    - identification methods and storage conditions

- the test conditions
    - date
    - technique
    - neutralisation medium

- the test results

The report should have a conclusion and identify the person responsible for testing.

7.2 PROPOSED LIMITS

7.2.1 QUANTITATIVE SPECIFICATION (minimum test portion is 1 g or 1 ml)

a. Category 1 products - specifically intended for use on babies and the eye area

Total viable count for aerobic mesophyllic micro-organisms: not more than 10^2 cfu/g or ml.

b. Category 2 products - other products

Total viable count for aerobic mesophyllic micro-organisms: not more than 10^3 cfu/g or ml.

It is important to note the interpretation of results.
The limit prescribed shall be interpreted as follows:

i) 10^2 - maximum limit of acceptance is 5×10^2

ii) 10^3 - maximum limit of acceptance is 5×10^3

7.2.2 QUALITATIVE SPECIFICATION

The following specific micro-organisms must not be detectable in a product sample of 0.1g or 0.1 ml:
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Candida albicans*

7.2.3 ACCEPTANCE CRITERIA

All samples will comply with the requirements of quantitative and qualitative specifications.

7.3 PROPOSED METHODS FOR MICROBIAL EXAMINATION OF COSMETIC PRODUCTS

Determination of the total viable mesophyllic aerobic count should be carried out according to the following factors:

- Precautions should be taken to avoid contamination of the product with micro-organisms which could be revealed in the test;
- Elimination of any antimicrobial properties of the product under analysis should be achieved by dilution, neutralisation or filtration;

- Evaluation of the number of viable aerobic mesophyllic micro-organisms will be carried out by using pour plate technique, surface plate technique or filtration. Enrichment methods are not required.
- Identification of specific micro-organisms will be carried out by using selective culture methods.

7.3.1 MATERIALS AND REAGENTS

a. Culture media and reagents

See Appendix (page 10).

b. Sample preparation

Microbiological examination should be carried out with a sample of 1 g or 1 ml minimum.

For products for which the weight is less than 1 g, collect together several samples in order to obtain 1 g or 1 ml.

Dissolve or dilute 1 g or 1 ml of the product in a validated neutralising diluent to obtain a 1 in 10 dilution. In case of poorly wettable substances, a suitable surface-active agent such as 0.1 per cent m/V of polysorbate 80 may be added.

If necessary, subsequent decimal dilutions can be obtained from the stock suspension, using the same diluent to quench the antimicrobial activities of the product or so that a colony count of 10 to 100 may be expected for easy counting.

7.3.2 EVALUATION OF THE NUMBER OF VIABLE AEROBIC MESOPHYLLIC MICRO-ORGANISMS

a. Membrane filtration

Use 2 filter membranes having a pore size not greater than 0.45 µm and whose effectiveness to retain bacteria has been established. The method described below assumes that membranes about 50 mm in diameter will be used.

Transfer the quantity representing 0.1 g of the product to be examined to each membrane and filter immediately. A subsequent rinse with a sterile wash solution may be an advantage to distribute the organisms evenly.

Transfer the membranes to the surface of the appropriate agar media. Incubate for bacteria at 30°C to 35°C for 3 days and for fungi at 20°C to 25°C for 5 days, unless a more reliable count is obtained in a shorter time.

Count the number of colonies which develop. Calculate the number of micro-organisms per gram or per millilitre of the product to be examined.

b. Plate count procedure

Using Petri dishes 9 to 10 cm in diameter, add to each dish, 1 ml of the prepared sample representing 0.1 g of the product and about 15 ml of appropriate molten agar medium at not more than 45°C. Mix and maintain on a cool horizontal surface until solidified.

Alternatively, spread the diluted product (usually 0.1 ml per plate representing 0.01 g of product) on the surface of the appropriate solidified media in a Petri dish of 9 to 10 cm diameter.

Incubate one plate at 30 to 35°C for 3 days for bacteria, and the other plate at 20 to 25°C for fungi for 5 days, unless a more reliable count is obtained in a shorter time.

Count the number of colonies which develop. Calculate the result using plates with the greatest number of colonies but not more than 300 colonies for bacteria and 100 colonies for fungi.

c. Expression of results

Count and record the number of colony forming units (cfu) for each plate. Calculate the total number of cfu per plate and multiply this number by the dilution rate to obtain the result in cfu per g or ml of product.

7.3.3 DETECTION OF SPECIFIC ORGANISMS

a. Specific micro-organisms

• *Pseudomonas aeruginosa*
On cetrimide medium (see appendix, page 10), typical colonies of *Pseudomonas aeruginosa* are flat, translucent and may look yellow greenish to blue.

At least the following confirmation tests shall be performed:
- gram stain
- oxidase test
- motility
- growth at 42°C

Pseudomonas aeruginosa is a gram (-) rod, motile, oxidase(+) and grows at 42°C.

Note: other additional relevant tests should be used to confirm the identifications.

• *Staphylococcus aureus*
On Baird-Parker medium (see appendix), typical colonies of *Staphylococcus aureus* appear black, shining, convex and are surrounded by a clear zone which may be partially opaque.

At least the following confirmation tests shall be performed:
- gram stain
- catalase test
- coagulase test

Gram (+) cocci, catalase (+), coagulase (+), may be considered as *Staphylococcus aureus.*

Note: other additional relevant tests should be used to confirm the identifications.

• *Candida albicans*
On Sabouraud-dextrose medium (see appendix), typical colonies of *Candida albicans* look white to beige, creamy and convex.

At least the following confirmation tests shall be performed:
- microscopic examination
- germ tube production test
- chlamydospores formation

Yeast demonstrating a germ tube (+) and giving chlamydospores, may be considered as *Candida albicans.*

Note: other additional relevant tests should be used to confirm the identifications.

b. Expression of results

Record results in the test report as presence or absence of specific micro-organisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans*).

APPENDIX

The following solutions and culture media should be used for the purposes for which they are prescribed. However, proprietary dehydrated culture media may alternatively be used provided they have identical nutritive and selective properties for the micro-organisms to be tested for.

1. DILUENT

Buffered sodium chloride-peptone solution pH 7.0.

Potassium dihydrogen phosphate	3.56 g	→ equivalent to 0.067M
Disodium hydrogen phosphate dehydrate	7.23 g	↗
Sodium chloride	4.30 g	
Peptone (meat or casein)	1.0 g	
Purified water	1000 ml	

Sterilise by heating in an autoclave at 121°C for 15 min.
Suitable neutralising agents may be added as necessary, e.g. polysorbate 20 or 80, lecithin, thiosulphate.

2. MEDIUM FOR DETERMINING BACTERIAL COUNT

Casein soy bean digest agar.

Pancreatic digest of casein	15.0 g
Papaic digest of soy bean	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.3 ± 0.2. Sterilise by heating in an autoclave at 121°C for 15 min.

3. MEDIUM FOR DETERMINING FUNGAL COUNT

Sabouraud-dextrose agar.

Peptones (meat and casein)	10.0 g
Dextrose monohydrate	40.0 g
Agar	15.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 5.6 ± 0.2. Sterilise by heating in an autoclave at 121°C for 15 min.

4. MEDIUM FOR THE DETECTION OF *PSEUDOMONAS AERUGINOSA*

Cetrimide agar.

Pancreatic digest of gelatine	20.0 g
Magnesium chloride	1.4 g
Dipotassium sulphate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Purified water	1000 ml
Glycerol	10.0 ml

Adjust the pH so that after sterilisation it is 7.2 ± 0.2. Sterilise by heating in an autoclave at 121°C for 15 min.

5. MEDIUM FOR THE DETECTION OF *STAPHYLOCOCCUS AUREUS*

Baird-Parker agar.

Pancreatic digest of casein	10.0 g
Beef extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Agar	20.0 g
Glycine	12.0 g
Sodium pyruvate	x10.0 g
Purified Water	950 ml

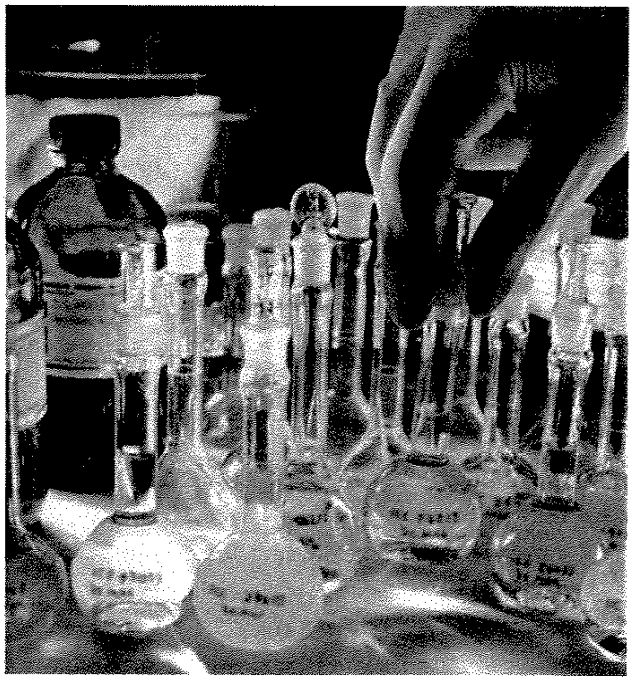
Adjust the pH so that after sterilisation it is 6.8 ± 0.2. Sterilise by heating in an autoclave at 121°C for 15 min., cool to 45 to 50°C and add 10 ml of a sterile 1% m/v solution of potassium tellurite and 50 ml of egg-yolk emulsion.

6. MEDIUM FOR THE DETECTION OF *CANDIDA ALBICANS*

Sabouraud-dextrose agar.

Peptones (meat and casein)	10.0 g
Dextrose monohydrate	40.0 g
Agar	15.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 5.6 ± 0.2. Sterilise by heating in an autoclave at 121°C for 15 min.



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